AMENDMENTS TO THE SPECIFICATION

1. Please replace the Title of the application on page 1, line 1 of the application (with reference to the PCT Publication) with the following replacement Title:

"Method and Kit for Primer Based Multiplex Amplification of Nucleic Acids Employing Primer Binding Tags"

2. Please replace the paragraph beginning on page 18 line 3 and ending on page 19, line 9 of the application (with reference to the PCT Publication) with the following replacement Paragraph:

FIG. 2 shows an embodiment of the direct detection method. Amplified nucleic acid (1) containing a target sequence (6) from a multiplex amplification reaction is provided for detection (the amplification process may be the Tem-PCR process described herein). Reporter oligonucleotides (2) comprising a hybridization domain (3) specific for a known target sequence (6) are provided and are conjugated to a means for first signal generation (4). The means for first signal generation is capable of producing a detectable first signal. The means for first signal generation (4) may be varied depending on the technology platform employed in the detection step. For example, if the LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) is employed as the technology platform in the detection step, the means for first signal generation (4) may be an internally color coded, spectrally addressable microsphere as shown in FIG. 2. Variations to the direct detection methods described below are described in co-pending U.S. application serial number 10/284,656, with the implementation of such variations being obvious to one of ordinary skill in the art.

3. Please replace the paragraph beginning on page 20 line 5 and ending on page 20, line 20 of the application (with reference to the PCT Publication) with the following replacement Paragraph:

The reporter oligonucleotides (2) are added to the denatured amplified nucleic acid (1) in appropriate hybridization buffer (such as 1X TMAC or 1X TE). The addition of the reporter oligonucleotides (2) may occur before or after denaturation (if employed). The reporter oligonucleotides (2) bind the target sequences (6) on the amplified nucleic acid (1) through the hybridization domain (3) forming a nucleic acid-reporter oligonucleotide complex (I). Hybridization conditions as are known in the art, such as by incubation at 52^0C for 15 minutes, may be used. After hybridization is complete, complex I is isolated, such as by centrifugation, and the unbound reporter oligonucleotides (2') and unbound amplified nucleic acid (1') are removed. Complex 1 may then be subject to detection using an appropriate detection platform. In one embodiment, the means for first signal generation (4) is an internally color coded spectrally addressable bead (obtained from Luminex Corporation, Austin, TX) and the ~~Luminex~~ LUMINEX® platform (a microsphere based multiplex detection system) is used for detection. The LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) stimulates the means for first signal generation (4) to produce a detectable first signal. The first signal is recorded and interpreted. The means for first signal generation (4) and the produced first signal are used to determine the identity of the target sequence (6) bound by the reporter oligonucleotide (2). Once the target sequence (6) has been identified, the identity of the disease agent or secondary disease agent detected is known.

4. Please replace the paragraph beginning on page 21 line 1 and ending on page 21, line 15 of the application (with reference to the PCT Publication) with the following replacement Paragraph:

The complex (II) may then be analyzed using an appropriate platform. In one embodiment, the means for first signal generation (4) is an internally color coded spectrally addressable bead (obtained from Luminex Corporation, Austin, TX) and the means for second signal generation (10) is a fluorescent PE label. In this embodiment,

the LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) is used for detection. The LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) stimulates the means for first (4) second (10) signal generation to produce a detectable first and second signal, respectively. The first and second signal generating means are selected such that the first and second signals can each be detected in presence of the other. The first and second signal are recorded and interpreted. The means for first signal generation and the produced first signal are used to determine the identity of the target sequence (6) bound by the reporter oligonucleotide (2). The means for second signal generation (10) and the second signal are used to confirm the presence of the target sequence (6) in combination with the reporter oligonucleotide (2) (to prevent signal generation from any free reporter oligonucleotide (2)). Once the target sequence (6a) has been identified, the identity of the disease agent or secondary disease agent detected is known. However, it should be noted that complex I may be subject to detection as described above

5. Please replace the paragraph beginning on page 22 line 2 and ending on page 22, line 14 of the application (with reference to the PCT Publication) with the following replacement Paragraph:

In one embodiment of the indirect detection protocol, a novel method termed ROCASH (Reporter Oligo Capturing After Specific Hybridization) is used. The ROCASH method is described in co-pending U.S. application serial number 10/284,656, which is hereby incorporated by reference as if fully set forth herein. For sake of clarity, one embodiment of the ROCASH method using the LUMINEX® ~~Luminex~~ X-Map technology is described (a microsphere based multiplex detection system). Other methods of indirect detection may also be used as is known in the art. It is understood that variations of the ROCASH method may be incorporated as described in the co-pending U.S. application serial number 10/284,656. Unlike the prior art methods, in the ROCASH method the specificity of hybridization between a reporter oligonucleotide and the target sequence and the sensitivity of the detection of the target sequence are provided

by different nucleic acid sequences of the reporter oligonucleotide hybridizing in different steps of the ROCASH method. Therefore, the conditions for these critical steps may be optimized independently of each other to provide for increased specificity and sensitivity in the detection step.

6. Please replace the paragraph beginning on page 22 line 15 and ending on page 22, line 24 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

The LUMINEX® ~~Luminex~~ xMAP technology (a microsphere based multiplex detection system) and related technologies are described in the art and in US Patent Nos. 6,524,473, 6,514,295, 6,449,562, 6,411,904, 6,366,354, 6,268,222, 6,139,800, 6,057,107, 6,046,807 and 5,736,330. The xMAP technology uses a plurality of internally color-coded microspheres covalently bound to target specific capturing reagents (termed cRTs, as defined below). Such capturing reagents may be oligonucleotides (as in the case of cRTs described below), but may also be polypeptides or chemical moieties designed to interact specifically with the region tags. When alternate capturing reagents are used, the region tags on the reporter oligonucleotides may altered to provide a complementary binding partner. The internal color coding generates a unique signal for each set of beads on excitation by the LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system).

7. Please replace the paragraph beginning on page 22 line 25 and ending on page 23, line 20 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

The ROCASH method may be considered to comprise two primary components: (i) a reporter oligonucleotide; and (ii) a means for collection. The reporter oligonucleotides are designed to bind specifically to the target sequence provided by the multiplex amplification step discussed above. The reporter oligonucleotides comprise a

hybridization domain of variable length designed to hybridize to the target sequence (see discussion above regarding the effect of the length of the hybridization domain on specificity and sensitivity), a nucleic acid sequence termed the region tag for hybridization to the means for collection, and a means for first signal generation. In one embodiment, the region tags on the reporter oligonucleotides are unique for each hybridization domain. In other words, a reporter oligonucleotide having a hybridization domain that binds to target sequence "A" and a reporter oligonucleotide having a hybridization domain that binds to target sequence "B" will have different region tags. The means for collection comprises and a plurality of capturing reagents, in this embodiment termed cRTs (complementary region tags) and a means for second signal generation. cRTs are nucleic acid sequences that are complementary to the region tags contained in the reporter oligonucleotides. The means for first and second signal generation may vary depending on the nature of the technology employed in the detection platform. When using the LUMINEX® ~~Luminex~~ X-Map technology (a microsphere based multiplex detection system), the means for first signal generation may be a fluorescent tag, such as PE or Cy-3, and the means for second signal generation may be an internally color coded, spectrally addressable microsphere. Through interaction of the region tags of the reporter oligonucleotide and the cRTs of the means for collection, each reporter oligonucleotide (which is specific for a known target sequence by virtue of the hybridization domain) will be associated with a known means for second signal generation (such as a color coded ~~Luminex~~ bead obtained from Luminex Corporation, Austin, TX). Therefore, by determining the identity of the means for second signal generation, the identity of the target sequence can be determined, which will allow the identification of the disease agent or secondary disease agent. Therefore, the specificity is achieved by the hybridization between the hybridization domain of the reporter oligonucleotide and the target sequence (note that specificity may be altered by decreasing or increasing the length of the hybridization domain) and the sensitivity is determined by the hybridization between the region tag of the reporter oligonucleotide and the cRT of the means for collection. A means for purification may also be used that

is designed to interact with the amplified nucleic acid sequences and aids in the removal of the unused amplified target nucleic acids as discussed below.

8. Please replace the paragraph beginning on page 24 line 27 and ending on page 25, line 3 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

In one embodiment, the means for first signal generation (4a) is a fluorescent PE label and the means for second signal generation (10a) is an internally color coded spectrally addressable bead (provided by Luminex Corporation, Austin, TX). In this embodiment, the LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) is used for detection. The LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) stimulates the means for first (4a) second (10a) signal generation to produce a detectable first and second signal, respectively. The first and second signal are recorded and interpreted. The first and second signal generating means are selected such that the first and second signals can each be detected in presence of the other. The means for second signal generation and the produced second signal are used to determine the identity of the target sequence (6a) bound by the reporter oligonucleotide (2a). The means for first signal generation (10a) and the second signal are used to indirectly confirm the presence of the target sequence (6a) (to prevent signal generation from any free means for collection 10a). Once the target sequence (6a) has been identified, the identity of the disease agent or secondary disease agent detected.

9. Please replace the paragraph beginning on page 24 line 27 and ending on page 25, line 3 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

An aliquot of the amplified products were subject to detection using the direct detection method as described above and illustrated in FIG. 2. In this example, detection oligonucleotides (as listed in Table 2) specific for a target sequence amplified in each

agent to be detected were coupled to ~~Luminex~~ microspheres (obtained from Luminex Corporation, Austin, TX). An aliquot of the amplified products were added, in separate tubes, to a reaction mixture containing 1X TE, hybridization buffer and an amount of each detection oligonucleotide sufficient to generate a detectable signal. In this example, detection oligonucleotide for each agent in Table 2 was present in each reaction containing the amplified nucleic acid products. For the detection oligonucleotide negative control, 1X TE was added in place of the amplified products. The samples were immediately placed at 52^0C for 15 minutes for hybridization between the detection oligonucleotide and the target sequence. The samples were centrifuged to remove unbound detection oligonucleotide and nucleic acid to which the detection oligonucleotides had not bound. The samples were then subject to detection using the LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system). The LUMINEX® ~~Luminex~~ platform (a microsphere based multiplex detection system) stimulated the microsphere conjugated to the detection oligonucleotide (the first signal generating means) to produce a detectable signal. In this example, a second means for detection was not included, however, a second signal may be incorporated as discussed above.

10. Please replace the paragraph beginning on page 29 line 21 and ending on page 29, line 23 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

The detection of the amplified products containing the target sequence was carried out as described for the direct detection methodology using ~~Luminex~~ beads as described in Example 1 (obtained from Luminex Corporation, Austin, TX). Detection oligonucleotides for each agent listed in Table 3 were added to each detection reaction.

11. Please replace the paragraph beginning on page 29 line 24 and ending on page 30, line 7 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

Table 6 shows the results of this experiment using the target enrichment and target amplification primers disclosed in Table 3. The rows represent the identity of the disease agent or secondary disease agent whose nucleic acid was used in the initial amplification reaction and the ratio of the primers used in the amplification step. The columns represent the detection oligonucleotides used in the detection step. In this example, sample number 1 is a RT-PCR Blank comprising a mixture of target enrichment and target amplification primers and detection oligonucleotides conjugated to ~~Luminex~~ beads (obtained from Luminex Corporation, Austin, TX) ~~was~~ were hybridized to the RT-PCR reaction that did not include template. The background signals are used to determine the cut-off values for a positive reaction. In a multiplexed system, such as the MAS utilizing TemPCR, each bead set is, in fact, a micro-system of its own. The final signal, as well as the background, is influenced by many factors including: the efficiency of the coupling reaction that links the capture oligonucleotides onto the bead sets; the efficiency of target amplification in the multiplexed TemPCR reaction; and the efficiency of hybridization during detection. As a result, the cutoff value for each pathogen (represented by each bead set) must be decided individually. The background signals (determined by averaging the values obtained from the RT-PCR blank and the samples that were known not to contain the target agent) were determined and the standard deviation obtained. The standard deviation was multiplied by five (5) and this value added to the average background. Values higher than the average background are considered positive results, indicating the presence of a particular agent.

12. Please replace the paragraph beginning on page 32 line 20 and ending on page 32, line 21 of the application (with reference to the PCT Publication) with the following replacement Paragraph.

The amplification conditions are given below. The reaction tubes were placed in a thermocycler programmed as follows: (i) reverse transcription- 30 minutes at 50^0C; (ii) initial PCR activation- 15 minutes at 95^0C; (iii) first amplification reaction comprising a first 3-step cycling (target enrichment)- 0.5 minutes at 94^0C, 1 minute at 52^0C and 1

minute at 72^{0}C for 15 complete cycles, and a second 2-step cycling (selective amplification)- 15 seconds at 94^{0}C, 1.5 minutes at 70^{0}C; for 6 complete cycles, preferably 4-8 complete cycles; (iv) second amplification reaction comprising a third 3-step cycling (target amplification)- 15 to 30 seconds at 94^{0}C, 15 to 30 seconds at 50-55^{0}C and 15 to 30 second at 72^{0}C; for at least 2 complete cycles, preferably for 10-40 complete cycles; and (v) final extension- 3 minutes at 72^{0}C. The detection of the amplified products containing the target sequence was carried out as described for the direct detection methodology using ~~Luminex~~ beads (obtained from Luminex Corporation, Austin, TX) as described in Example 1 and illustrated in FIG. 2. Detection oligonucleotides for each agent listed in Table 3 were added to each detection reaction.

13. Please replace Table 8 on pages 42-43 of the application (with reference to the PCT Publication) with replacement pages 42, 43 and 43a. The Replacement pages are attached as Appendix A following page 16 of this paper.

14. Please replace the Tables 2 and 3 on pages 35-39 of the application as filed with replacement pages 35-59. The Replacement pages are attached as Appendix B following page 16 of this paper.